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The prevalence of hrHPV in a significant cohort of Romanian women

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Abstract

The aim of this study was to analyze the prevalence of hrHPV correlated to cytological abnormalities in a significant cohort of women from Romania. ***Methods:*** *The study group included 625 Romanian female patients aged 16 to 68 years that were referred to Synevo laboratories for performing both a liquid-based Papanicolaou test and a high-risk HPV DNA test.* ***Results****: Cytological abnormalities were presented in 199 patients (31.84%) and HPV infections were detected positive in 215 patients (34.4%). When considering all 215 HPV positive samples, 13.02% of samples were associated with HSIL, 27.91% with LSIL, 31.63%, with ASC-US and 6.98% ASC-H. However, 20.47% from the samples presenting HPV DNA were included in NILM group. The prevalences of hrHPV were as follows: HPV16 27.82%; HPV18 7.26%, other hrHPV 64.92%. Analysis per cytological groups revealed that HPV 16 had a similar prevalence in LSIL and HSIL (34.29 vs. 34.48%) compared to HPV 18 that had the highest prevalence in ASC-H (27.78%). Other pooled hrHPV registered high prevalence in all abnormal cytologies (between 55.56% and 65.85%). Stratification by age showed a little variation of HPV16 and HPV18 prevalences in all age groups.* ***Conclusion****. Considering the high prevalence of hrHPV infections in Romanian women the support for vaccination programs and an increased availability of clinically validated HPV molecular tests are necessary.*

Introduction

Cervical cancer is the fourth most frequent cancer in women all over the World and the second most common female cancer in the 16-44 years age group in Europe (BRUNI & al. [1]). According to statistics provided by GLOBOCAN 2012, 528000 new cases of cervical cancer and 266000 deaths are estimated annually. Developing countries account for more than 85% of new cervical cancer cases. The overall incidence of cervical cancer is growing, being expected an increase of 42% by the year 2020 (FERLAY & al. [2]). In Europe there is an unequal distribution of incidence and mortality rates per 100,000 population among different regions. Thus, in Eastern Europe the incidence and mortality rates are 16.3% and 6.2% compared to Western Europe countries where rates of 7.3% and respectively 1.8% are reported (BRUNI & al. [1]). Romania has the highest rate of incidence and mortality in Europe, a situation which has been constant since early 1980. Cervical cancer affects young, active women and is the leading cause of death for the Romanian female population belonging to the age category 20-44 years. (JSI RESEARCH &TRAINING INSTITUTE, INC./ROMANIAN FAMILLY HEALTH INITIATIVE FOR THE USA AGENCY FOR INTERNATIONAL DEVELOPMENT [3]). Between 2000 - 2006 there were reported 22830 new cases of cervical cancer and 1273 deaths. In 2005 the incidence rate ranged

between 17.8-31.2 and mortality between 12.3 - 21.5 (APOSTOL & al. [4]). In 2012 the age-standardized incidence rate of cervical cancer in Romania was estimated to 28.6% (FERLAY & al. [2]).

Persistent infection with high-risk (oncogenic) human papilloma virus (hrHPV) genotypes is the most important risk factor for the occurrence of premalignant lesions and cervical cancer, being a necessary, although not sufficient condition (ROSITCH & al. [5]). Other risk co-factors such as smoking, prolonged consumption (>6 years) of oral contraceptives, persistent immunosuppression, other sexually transmitted diseases and sexual behavior might play a role in cervical carcinogenesis (BURCELL & al. [6]). Based on their involvement in cervical cancer, HPV genotypes were classified into the following groups: HPV types with high oncogenic risk or carcinogenic (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59); HPV types probably carcinogenic (68); HPV types possibly carcinogenic (26, 53, 66, 67, 70, 73, 82); HPV types with low oncogenic risk (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108 (BOSCH & al. [7]; MARKOWITZ & al. [8]; SCHIFMANN & al. [9]). Virtually all cases of cervical cancer are caused by oncogenic HPV genotypes; among them, HPV16 and 18 are responsible for about 70% of all cases (SASLOW & al [10]). Although the progression to cancer is rare, the high prevalence of HPV infection among the population explains why the cancers related to HPV, including the cervical cancer and other anogenital cancers, are among the most frequent malignancies (DUNNE & MARKOWITZ [11]).

In order to prevent morbidity and mortality related to cervical cancer several strategies of screening have been attempted in the course of time, their aim being to detect cervical lesions that can progress to cancer. Adopting cervical cytology in Papanicolaou (Pap) smears as an effective mass screening method have significantly reduced cervical cancer incidence in the developed countries. However, important limitations of cytology have been identified, such as low sensitivity (50-60%) due to pre-analytical issues and subjective interpretation (COX & CUZICK [12]; SASLOW & al. [10]). Introduction of HPV testing in clinical settings aimed to improve the rate of cervical premalignant lesion detection (SASLOW & al. [10]).

There are only few Romanian studies concerning the prevalence of HPV infections and their association with cervical lesions, especially in ambulatory units. In this respect, the purpose of our study was to analyze the prevalence of hrHPV correlated to cytological abnormalities in a significant cohort of women that performed liquid-based cytology combined to a hrHPV test featured to detected clinically relevant infections.

Materials and methods

Patients

The study group included 625 Romanian female patients aged 16 to 68 years that were referred to Synevo laboratories between October and December 2014 for performing both a liquid-based Papanicolaou test (BD SurePath™ liquid-based Pap test, Becton Dickinson) and a high-risk HPV DNA test (Cobas® 4800 Human Papillomavirus, Roche Molecular Diagnostics). The combined testing was recommended predominantly as a part of a routine control (70.77 % of the cases), or due to various medical conditions: cytological abnormalities detected during the last 3-12 months (16.93%), cervicitis (5.75%), recent surgery for cervical intraepithelial lesions (CIN, 5.11%), genital condylomatosis (0.80%), previous HPV infections without cytological abnormalities (0.64%).

After obtaining the informed consent from the patients, cervical samples were collected by specialized nurses using a soft cervical brush (Cervex-Brush®, Rovers Medical Devices B.V., Oss - The Netherlands) that allows simultaneously sampling of ectocervical, endocervical and

transformation-zone cells for cervicovaginal cytology as well as for HPV test. Following sample collection, the detachable head of the brush was placed into the SurePath vial (Becton Dickinson) that provides efficient cell preservation and a proper transportation medium.

The liquid-based cytology

The liquid-based cytology was performed on Tripath platform (Becton Dickinson) including several sample processing steps: vial vortexing, centrifugal sedimentation through a density reagent (to partially remove non-diagnostic debris and excess inflammatory cells and to ensure sample enrichment with clinically relevant cells), automatic slide preparation and staining. The slides prepared in triplicate for each sample were examined under microscope by qualified cytopathologists using a digital image processing system (BD FocalPoint™ GS Imaging System). Results were reported according to the Bethesda 2001 guidelines.

HPV detection

HPV DNA test was performed on the automatic platform Cobas 4800 (Roche Molecular Diagnostics) using a qualitative Real-time PCR assay that detects 14 high-risk HPV clinically relevant genotypes. This test provides concurrently individual results for the highest risk genotypes, HPV 16 and 18, and pooled results for the other 12 high-risk HPV genotypes (HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). Target DNA is a sequence of approximatively 200 bp located within the polymorphic L1 region of HPV genome. Human β-globin gene serves as an internal control, monitoring the whole test process.

Cervical samples were automated prepared to extract HPV as well as cellular DNA followed by amplification of target DNA and β-globin gene using specific primer pairs and real-time detection of the cleaved oligonucleotide probes that are labeled with four fluorescent dyes corresponding to the 12 high-risk HPV genotypes, HPV16, HPV 18, and β-globin gene. According to manufacturer instructions a set of negative and positive controls were used in each run in order to validate the results.

Statistical analysis

Frequencies and percentages were used to describe qualitative data while for quantitative data were used the range (minimum and maximum), mean, and standard deviation. The data were stratified by age (≤ 20 years, 21-25 years, 26-30 years, 31-35 years, 36-40 years, 41–45 years, 46–50 years and >50 years). For association between HPV infection in cervical lesions with cytology logistic regression analysis was performed. Data were analyzed by Student's t test and different categorical variables were compared by two ways ANOVA with Bonferroni posttests.

Results

Of 625 patients referred to Synevo laboratories, 199 patients (31.84%) presented cytological abnormalities with the following distribution according to the type of cervical lesions: 4.64% high-grade squamous intraepithelial lesions (HSIL), 11.2% low-grade squamous intraepithelial lesions (LSIL), 13.12% atypical squamous cells of undetermined significance (ASC-US), and 2.88% atypical squamous cells- HSIL cannot be excluded (ASC-H).

By employing Cobas HPV assay that is able to identify clinically relevant HPV infections we detected positive results in 215 patients (34.4%). When considering all HPV positive samples (215 patients), 13.02% of samples were associated with HSIL, 27.91% with LSIL, 31.63%, with ASC-US and 6.98% ASC-H. However, 20.47% from the samples presenting HPV DNA were

included in NILM group. According to figure 1, the highest prevalence of HPV infection was observed in HSIL category (96.55%), while the lowest one was found in NILM patients (10.32%) ($p<0.002$). The other cytological abnormalities, ASC-US, ASC-H and LSIL were also associated with a high HPV prevalence: 82.93%, 83.33% and respectively, 85.71%. The overall prevalence of hrHPV in women with abnormal cytologies was 85.92%.

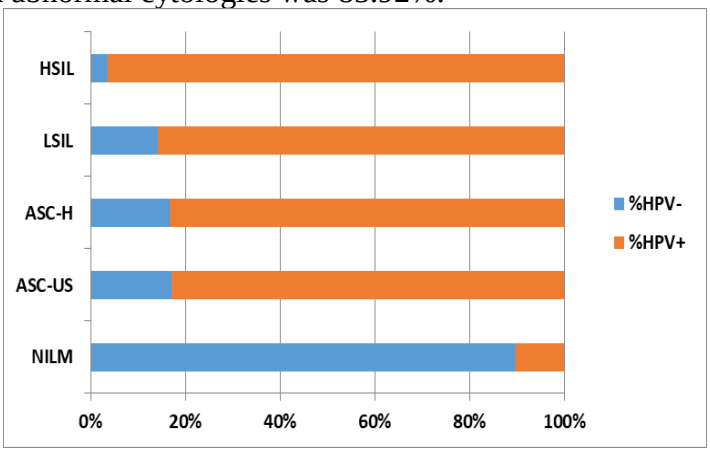

Fig. 1. The percent of hrHPV+ in cytological groups.

Taking into account that age is a critical factor when assessing the relation between HPV infections and cervical cancer, the patients were furtherly stratified into 5 year age groups, starting with less than 20 years and ending with over 50 years. The prevalence of hrHPV infection progressivelly increased reaching maximum value in 26-30 years group and then registered a gradual decrease with age. Also, a similar pattern of abnormal cytologies in relation to age groups was observed (r=0.988) (figure 2).

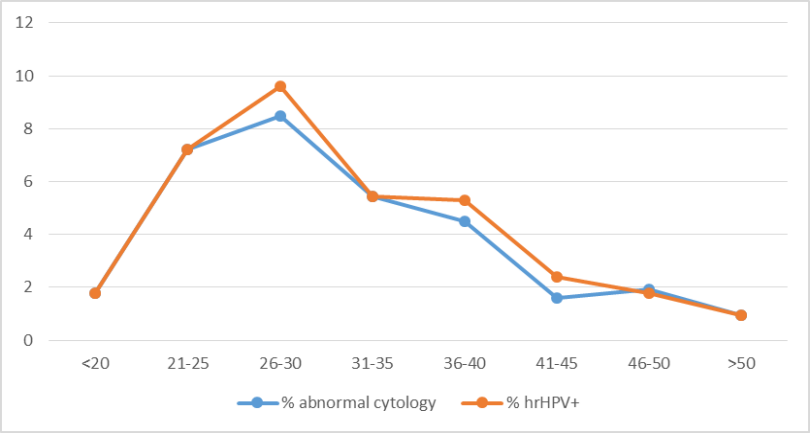

Fig. 2. Prevalence of hrHPV infection and abnormal cytologies related to age groups (all 625 samples were considered).

When considering each age group separately we found that in women less than 25 years the prevalence of hrHPV was highest in LSIL and ASC-US cytology. In patients over 46 years, the presence of hrHPV was most frequently associated with HSIL cytology. The highest percent of HPV negative samples were associated with NILM cytology and older patients (table 1). Analysis of all hrHPV positive samples per age group revealed a similar profile of increased association with LSIL in younger patients (figure 3).

Table 1. Prevalence of HPV in cervical lesions in each age group.

		NILM	ASC-US	ASC-H	LSIL	HSIL
<20	HPV(-)	43.48% (n=10)	4.35% (n=1)	(n=0)	4.35% (n=1)	(n=0)
	HPV+	8.70% (n=2)	13.04% (n=3)	(n=0)	26.09% (n=6)	(n=0)
21-25	HPV(-)	46.15% (n=42)	2.20% (n=2)	(n=0)	2.20% (n=2)	(n=0)
	HPV+	4.40% (n=4)	15.38% (n=14)	4.40% (n=4)	18.68% (n=17)	6.59% (n=6)
26-30	HPV(-)	54.55% (n=78)	2.10% (n=3)	(n=0)	1.40% (n=2)	(n=0)
	HPV+	8.39% (n=12)	16.08% (n=23)	3.50% (n=5)	9.09% (n=13)	4.90% (n=7)
31-35	HPV(-)	62.75% (n=64)	2.94% (n=3)	(n=0)	0.98% (n=1)	0.98% (n=1)
	HPV+	4.90% (n=5)	9.80% (n=10)	1.96% (n=2)	9.80% (n=10)	5.88% (n=6)
36-40	HPV(-)	61.76% (n=63)	2.94% (n=3)	1.96% (n=2)	0.98% (n=1)	(n=0)
	HPV+	10.78% (n=11)	6.86% (n=7)	1.96% (n=2)	9.80% (n=10)	2.94% (n=3)
41-45	HPV(-)	76.06% (n=54)	(n=0)	(n=0)	2.82% (n=2)	(n=0)
	HPV+	9.86% (n=7)	7.04% (n=5)	(n=1)	4.23% (n=3)	(n=0)
46-50	HPV(-)	72.92% (n=35)	(n=0)	(n=0)	2.08% (n=1)	(n=0)
	HPV+	(n=0)	10.42% (n=5)	4.17% (n=2)	2.08% (n=1)	8.33% (n=4)
> 50	HPV(-)	80.00% (n=36)	4.44% (n=2)	2.22% (n=1)	(n=0)	(n=0)
	HPV+	6.67% (n=3)	2.22% (n=1)	(n=0)	(n=0)	4.44% (n=2)

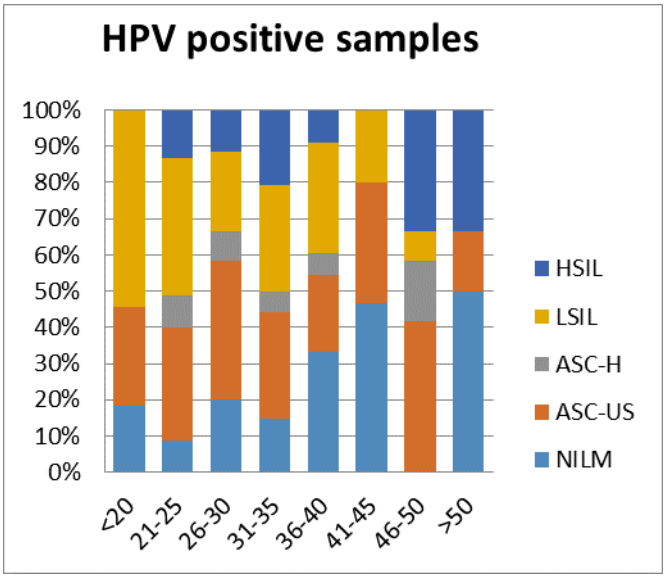

Fig. 3. Distribution of cytological abnormalities per age groups in hrHPV positive samples.

As Cobas HPV assay provides individual genotyping information for HPV16 and HPV18 and reports all other 12 hrHPV as a pool we analysed in the next step the distribution of HPV genotypes in all HPV positive samples, within each cytological group and per age groups. Thus, considering all HPV positive samples, the prevalences were: HPV16 27.82%; HPV18 7.26%, other hrHPV 64.92%. Analysis per cytological groups revealed that HPV 16 had a similar prevalence in LSIL and HSIL (34.29 vs. 34.48%) compared to HPV 18 that had the highest prevalence in ASC-H (27.78%). Other pooled hrHPV registered high prevalence in all abnormal cytologies (between 55.56% and 65.85%) (Figure 4). Stratification by age showed a little variation of HPV16 and HPV18 prevalences in all age groups with a slight increase in women over 50 years (figure 5). Multiple HPV infections (confirmed through detections of at least tow fluorescent signals), were detected in 33 hrHPV DNA positive cases (15.3%), being more prevalent in younger women with ASC-US and LSIL cytology.

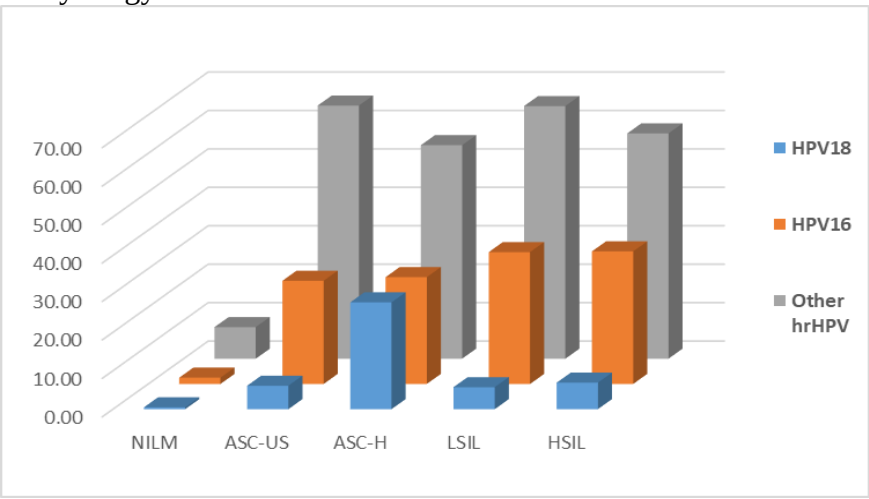

Fig.4. hrHPV genotypes distribution per cytological group

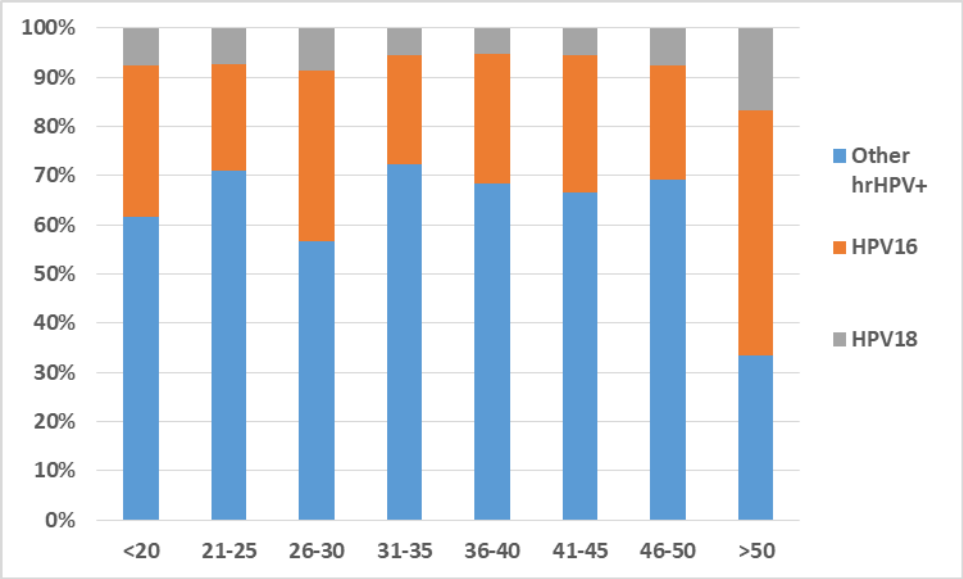

Fig. 5. hrHPV genotypes distribution per age group

Discussion and future perspectives

The results of our study indicate a 34.4% prevalence of HPV infection with oncogenic genotypes that is closely correlated with the prevalence of cytological abnormalities (31.84%) among the population referred for co-testing to the Synevo laboratories from different geographical areas of Romania. The correlation has been observed also when we analyzed the prevalence by age-groups. These data support the good clinical performance of Cobas HPV assay that was previously validated by ATHENA (Addressing THE Need for Advanced HPV Diagnostics) study, the test being able to identify women at highest risk of developing premalignant lesions (CASTLE & al. [13]; WRIGHT & al. [14]).

The prevalence of hrHPV genotypes is slightly increased compared to the prevalence reported by the largest Romanian study that included 1000 women from Brasov county: 29%. Moreover, the prevalence of hrHPV in women with NILM (10.32%) is much lower and prevalence in cases with abnormal cytologies is much higher (85.92%) than in the above mentioned study: 24.2% and 48.24%, respectively (MOGA & al. [15]). These differences can be partly explained by the type of HPV assay employed - Cobas HPV versus LINEAR ARRAY HPV Genotyping Test.

When considering the prevalence of hrHPV genotpyes per age group, we found an obviously higher prevalence in women aged ≤ 25 years (49.1%) compared to women above 40 years (19.6%). While in younger women the hrHPV genotypes was mostly associated with ASC-US and LSIL cytology, in elder women the association of hrHPV was stronger with HSIL cytology. These data suggest a low progress of HPV infection acquired during early ages (MELON & al. [16]).

In our study, HPV 16 genotype was detected in 27.82% of all hrHPV DNA positive samples. Other Romanian studies reported the following HPV 16 genotype prevalence: 24.7% (Moga et al., 2014), 29.8% (URSU & al. [17]) and 34.7% (ANTON & al. [18]), indicating there might be some regional variations in hrHPV genotypes distribution.

In contrast with previously other Romanian studies,we found a higher prevalence of hrHPV genotypes and particularly HPV 16 genotype in LSIL cytology (85,71% and 34,28%, respectively). As the risk of LSIL progression is dependent on the certain hrHPV genotype(s), being significantly higher in women carrying HPV 16 (MATSUMOTO & al. [19]), these data suggest an increased risk of cervical precursor lesion persistance and progression in LSIL women from our group. In order to avoid unnecessary invasive procedures (colposcopy and biopsy) and treatment in LSIL that is mostly prevalent in women under 30 years, immunocytochemical dual staining of p16/Ki-67 was proposed as an additional test for cervical cancer screening, being able to predict with high accuracy the high-grade cervical intraepithelial neoplasia in women ≤30 years diagnosed with LSIL (POSSATI-RESENDE & al. [20]).

As a limitation of our study, the results of the cervical biopsies performed on the female patients with cytological abnormalities were not available; these would have enabled a better correlation of hrHPV genotypes with cervical lesions.

Considering the high prevalence of oncogenic HPV infections in Romanian women with abnormal cytologies, both the support for vaccination programs and an increased availability of clinically validated HPV molecular tests are necessary.

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